contained restriction sites to allow the in-frame fusion with the Cub-RURA3 module located in the vector pRS305 (Sikorski and Hieter, 1989). The short linker sequence between the last codon of SEC63 and the first codon of Cub reads: **GAA** GGC GGG TCG ACC **GGT** (SEQ ID NO: 2). The last codon of SEC63 and the first codon of Cub are in bold letters; the SalI site is underlined. The vector was cut at its unique PstI site in the SEC63-containing fragment and transformed into the S. cerevisiae strains JD51 and JD55 to yield, through homologous recombination, the integrated cassette that expressed Sec63-Cub-RUra3p from the native promoter of SEC63 and a short C-terminal fragment of SEC63 comprising its last 448 bp. Integration was confirmed by PCR. SEC63-Cub-Dha was created in a similar manner. The linker between SEC63 and the Cub-Dha module reads: **GAA** GGC GGG TCG ACC ATG TCG GGG **GGG** (SEQ ID NO: 3). The last codon of SEC63 and the first codon of Cub are printed in bold letters. The Cub-Dha module is described by Johnsson and Varshavsky (1994). FUR4-Cub-RURA3 was created similar to SEC63-Cub-RURA3. The PCR product containing the last 952 bp of the ORF of the FUR4 gene were inserted in front of the Cub-RURA3 module located in the pRS303 vector using an EagI and a SalI site at the ends of the PCR product. The linker between the last codon (bold letters) of FUR4 and the first codon of Cub (bold letters) reads: **ATT** GGG TCG ACC **GGT** (SEQ ID NO: 4). The SalI site is underlined. The vector was cut at the unique EcoRI site in the FUR4-derived fragment to create, through homologous recombination, a C-terminal fragment of the gene of 955 bp and the integrated cassette that expressed Fur4-Cub-RUra3p from the FUR4 promoter. Integration was confirmed by PCR. Two nucleotide exchanges were found in the FUR4 PCR product when compared with the corresponding sequence in the yeast genome database leading to an Asp and Glu in position 421 and 617 of the Fur4p-construct instead of the Asn and Val encoded in the genomic sequence. Since Fur4p-Cub-RUra3p still conferred 5-fluoroorotic acid (5-FOA) sensitivity to the transformed yeast, we inferred that the Cub construct is functional. STE14-Cub-RURA3 was constructed using two primers to amplify the complete ORF of STE14 using genomic DNA as a template. The PCR product was inserted between the Cub-RURA3 module and the P_{MET25}-promoter in the vector pRS315. The linker between the last codon (bold letters) of STE14 and the first codon of Cub (bold letters) reads: **ATA** GGG TCG ACC **GGT** (SEQ ID NO: 5). The SalI site is underlined. The same PCR product was inserted between the P_{GAL1}-promoter and Dha to create STE14-Dha in the pRS314 vector. The sequence between the last codon of STE14 and Dha reads: ATA GGG TCG ACC TTA ATG CAG AGA TCT GGC ATC ATG GTT (SEQ ID NO: 6). The last codon of STE14 and the first two codons of Dha are underlined. The sequence connecting the last codon of

SEC62 (underlined) and Dha of SEC62-Dha in pRS314 reads: AAC GGC GGG TCG ACC TTA ATG CAG AGA TCT GGC ATC ATG GTT (SEQ ID NO: 7). TOM20-Cub-RURA3 was constructed similar to STE14-Cub-RURA3. The PCR product was inserted between the PCUP1-promoter and the Cub-RURA3 module in the vector pRS315. The linker between the last codon of TOM20 (bold letters) and the first codon of Cub (bold letters) reads: **GAC** GGG TCG ACC **GGT** (SEQ ID NO: 8). The SalI site is underlined.

- On Pages 118 and 119, please replace the second and first partially complete paragraphs respectively with the following text:

The Nub-constructs were assembled from the P_{CUP1}-Nub-cassette and a PCR fragment containing the ORF or part of the ORF of the desired gene to finally reside in the vector pRS314, pRS313, or pRS304. A BamHI site was used to bring the Nub in frame with the PCR product. The linker between the last codon of Nub (bold letters) and the first codon of the following ORF (bold letters) reads: GG ATC**CCT** GGC **GTC** (SEQ ID NO: 9) for TOM22, GG ATC**CCT** GGG TCT GGG **ATG** (SEQ ID NO: 10) for SEC61 and SSH1, GG ATC **CCT** GGG GAT **ATG** (SEQ ID NO: 11) for SNC1, SSO1, TPI1, GUK1, GG ATC **CCT** GGG GAT **TCC** (SEQ ID NO: 12) for VAM3. The BamHI site is underlined. Nub-SEC61 was constructed by targeted integration of a Nub-SEC61-containing fragment into SEC61 of the S. cerevisiae strain JD53. A fragment containing the first 875 bp of the SEC61 ORF was amplified by PCR and inserted downstream of the pRS304- or pRS303-based P_{CUP1}-Nub cassette, using the flanking BamHI and EcoRI sites. For targeted integration, the plasmid was linearized at the unique StuI site in the SEC61 ORF to create the yeasts NJY61-I, -A, and -G. Integration was confirmed by PCR. To construct Nub-Ssh1p, a fragment of 680 bp was amplified by PCR and inserted downstream of the pRS304-based P_{CUP1}-Nub cassette using the flanking BamHI and XhoI sites. The vector was cut for targeted integration at the unique ClaI site in the SSH1 ORF to create the yeast strains NJY78-I, -A, -G, and -VI. Integration was confirmed by PCR. The construction of Nub-SEC62, -SED5, -STE14, and -BOS1 was described in Dünnwald et al. (Mol. Biol. Cell 10: 329-344, 1999). The functionality of Nub-Sed5p and -Sec62p was confirmed by complementing a yeast strain carrying a ts mutation in the corresponding gene. Nub-Sso1p, Nub-Guk1p, and Nub-Tpi1p were shown to support growth of S. cerevisiae cells under conditions where the corresponding, unmodified protein was not expressed. Nub-Snc1p, -Tom22p, -Vam3p, and -Ssh1p were not tested. The functionality of Nub-Sec61p in the strain NJY61-I was tested by repeating the transformation of JD53 with a StuI cut vector bearing a shift in the reading frame between Nub

and SEC61. As a consequence, no full-length Sec61p should be expressed in the transformed haploids, but only the N-terminal fragment from the first 875 bp of the SEC61 ORF. Viable haploids would document that the N-terminal fragment of Sec61p can substitute for the full-length protein. However, the occasional colonies that were obtained after transformation were shown by PCR to always harbor a native SEC61 in addition to the modified Nub-SEC61 allele carrying the frame shift between the Nub and the SEC61 ORF. This shows that in the strain NJY61-I, the essential function of Sec61p was contributed by Nub-Sec61p.

➢ On Page 120, please replace the third complete paragraph with the following text:

The open reading frame of STE14 was replaced by the dominant kan^r marker essentially as described by Güldener et al. (1996). The PCR primers used for the construction of the kan^r disruption cassette were 5'- CCCCCTCTTTCATTGTGGTCACCGTTTTTGAAC ACAACCAGCTGAAGCTTCGTACGC (SEQ ID NO: 13) and 5'-CACAAAAATCCAGTCCATAACTAACACAATCATTACTAGCATAGGCCACTAGGTG ATCTG (SEQ ID NO: 14). Underlined are the sequences immediately preceding the ATG or following the stop codon of the coding sequence of STE14 (Sapperstein et al., 1994). Transformed yeast cells were selected for kan^r integration by Geneticin (Life Technologies, Paisley, Scotland), and the deletion was verified by diagnostic PCR and the mating deficiency of the cells.

➢ On Page 138, please replace the first complete paragraph with the following text:

The *S. cerevisiae* strains used were JD52, JD53, JD55, and NLY2. The NHP6 deletion strains were made by successive deletion of the entire NHP6A and NHP6B ORFs with the help of two knockout constructs based on NKY51. After each knockout, the URA3 gene was recombined out on 5-fluoroorotic acid (FOA) plates, and the hisG fragment remained in the place of the NHP6A and NHP6B ORFs. Consistent with previous reports, NHP6 deletion from JD52, JD53, and NLY2 caused temperature sensitivity. The NHP6 deletions were complemented by the integrative plasmids ASZ10 and YIplac128 containing PCR fragments of the NHP6A or NHP6B genes, respectively. The TUP1 deletion strains were constructed by first deleting the ADE2 gene of JD52 and JD53. An ADE2-marked PCR fragment containing 60 base pairs of the promoter and terminator sequences of TUP1 was then used to delete the entire TUP1 ORF. The REG1 deletion strains were generated by deleting the entire REG1 ORF with a HIS3-marked knockout vector. Genomic DNA was isolated from all *S. cerevisiae* knockout strains, and the deletions of

the respective genes were verified by PCR and Southern blotting. The *Escherichia coli* strain used for protein purification was BL21(DE3)LysS (Stratagene). The single-copy C_{ub}-RUra3p fusion vector has been described previously. The N_{ub} fusion vectors PACNX-N_{ub}IBC and PADNX-N_{ub}IBC are single-copy and multicopy derivatives of PADNS. In these vectors, we replaced the ampicillin resistance gene with the chloramphenicol resistance gene and subcloned a PCR fragment encoding the N-terminal half of ubiquitin, a hemagglutinin (HA) tag, and a *Bgl*II site in all three reading frames under the control of the ADH1 promoter. The oligonucleotides used are: GCCAAGCTTATGCAGATTTTCGTCAAGAC (SEQ ID NO: 15), GCCAGATCTCCAGCGTAATCTGGAACA (SEQ ID NO: 16), GCCAGATCTgCCAGCGTAATCTGGAACA (SEQ ID NO: 17), and GCCAGATCTggCCAGCGTAATCTGGAACA (SEQ ID NO: 18). The single-copy C_{ub}-RGFP fusion vector was constructed by replacing the *Mscl*/*Apa*I fragment containing the URA3 gene of the C_{ub}-RUra3p fusion vector with a *Stu*I/*Apa*I PCR fragment containing the DNA encoding the green fluorescent protein (GFP). The oligonucleotides used here are GCCAGGCCTCATGAGTAAAGGAGAAGAACT (SEQ ID NO: 19) and GCCGGGCCCTATTTGTATAGTTCATCCATGC (SEQ ID NO: 20). Following standard procedures, we generated the different fusions by cloning PCR fragments of the respective genes into the C_{ub} and N_{ub} fusion vectors. The glutathione *S*-transferase (GST)-Nhp6B fusion was made by cloning the NHP6B ORF into GEX-5X-1 (Amersham Pharmacia). H_6HA-Tup1p was constructed by cloning a PCR fragment containing the TUP1 ORF, six histidines, and an HA tag into pET11a (Invitrogen).

The replacement paragraphs presented above incorporate changes as indicated by the marked-up versions below.

The Cub-RUra3 reporter module was constructed by PCR amplification. The fragment covered residues 35-76 of UBI4 and a SalI and BamHI site to bring the fragment in front of the LACI-URA3 gene fusion (Ghislain et al., 1996). The sequence between the C terminus of Cub and the LACI sequence of the RURA3 reads: **GGT GGT AGG** CAC GGA TCC (SEQ ID NO: 1). The last two residues of the Cub and the N-terminal arginine of the RURA3 are printed in bold letters; the BamHI site is underlined. SEC63-Cub-RURA3 was constructed by PCR amplification of the last 445 base pairs (bp) of the coding sequence of SEC63 not including the stop codon by using genomic DNA of S. cerevisiae as a template. The ends of the PCR product contained restriction sites to allow the in-frame fusion with the Cub-RURA3 module located in

the vector pRS305 (Sikorski and Hieter, 1989). The short linker sequence between the last codon of SEC63 and the first codon of Cub reads: **GAA** GGC GGG TCG ACC **GGT** (SEQ ID NO: 2). The last codon of SEC63 and the first codon of Cub are in bold letters; the SalI site is underlined. The vector was cut at its unique PstI site in the SEC63-containing fragment and transformed into the S. cerevisiae strains JD51 and JD55 to yield, through homologous recombination, the integrated cassette that expressed Sec63-Cub-RUra3p from the native promoter of SEC63 and a short C-terminal fragment of SEC63 comprising its last 448 bp. Integration was confirmed by PCR. SEC63-Cub-Dha was created in a similar manner. The linker between SEC63 and the Cub-Dha module reads: **GAA** GGC GGG TCG ACC ATG TCG GGG **GGG** (SEQ ID NO: 3). The last codon of SEC63 and the first codon of Cub are printed in bold letters. The Cub-Dha module is described by Johnsson and Varshavsky (1994). FUR4-Cub-RURA3 was created similar to SEC63-Cub-RURA3. The PCR product containing the last 952 bp of the ORF of the FUR4 gene were inserted in front of the Cub-RURA3 module located in the pRS303 vector using an EagI and a SalI site at the ends of the PCR product. The linker between the last codon (bold letters) of FUR4 and the first codon of Cub (bold letters) reads: **ATT** GGG TCG ACC **GGT** (SEQ ID NO: 4). The SalI site is underlined. The vector was cut at the unique EcoRI site in the FUR4-derived fragment to create, through homologous recombination, a C-terminal fragment of the gene of 955 bp and the integrated cassette that expressed Fur4-Cub-RUra3p from the FUR4 promoter. Integration was confirmed by PCR. Two nucleotide exchanges were found in the FUR4 PCR product when compared with the corresponding sequence in the yeast genome database leading to an Asp and Glu in position 421 and 617 of the Fur4p-construct instead of the Asn and Val encoded in the genomic sequence. Since Fur4p-Cub-RUra3p still conferred 5-fluoroorotic acid (5-FOA) sensitivity to the transformed yeast, we inferred that the Cub construct is functional. STE14-Cub-RURA3 was constructed using two primers to amplify the complete ORF of STE14 using genomic DNA as a template. The PCR product was inserted between the Cub-RURA3 module and the P_{MET25}-promoter in the vector pRS315. The linker between the last codon (bold letters) of STE14 and the first codon of Cub (bold letters) reads: **ATA** GGG TCG ACC **GGT** (SEQ ID NO: 5). The SalI site is underlined. The same PCR product was inserted between the P_{GAL1}-promoter and Dha to create STE14-Dha in the pRS314 vector. The sequence between the last codon of STE14 and Dha reads: ATA GGG TCG ACC TTA ATG CAG AGA TCT GGC ATC ATG GTT (SEQ ID NO: 6). The last codon of STE14 and the first two codons of Dha are underlined. The sequence connecting the last codon of SEC62 (underlined) and Dha of SEC62-Dha in pRS314 reads: AAC GGC GGG TCG ACC TTA

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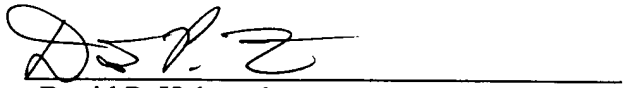
site in all three reading frames under the control of the ADH1 promoter. The oligonucleotides used are: GCCAAGCTTATGCAGATTTTCGTCAAGAC (SEQ ID NO: 15), GCCAGATCTCCAGCGTAATCTGGAACA (SEQ ID NO: 16), GCCAGATCTgCCAGCGTAATCTGGAACA (SEQ ID NO: 17), and GCCAGATCTggCCAGCGTAATCTGGAACA (SEQ ID NO: 18). The single-copy C_{ub}-RGFP fusion vector was constructed by replacing the *MscI/ApaI* fragment containing the URA3 gene of the C_{ub}-RUra3p fusion vector with a *StuI/ApaI* PCR fragment containing the DNA encoding the green fluorescent protein (GFP). The oligonucleotides used here are GCCAGGCCTCATGAGTAAAGGAGAAGAACT (SEQ ID NO: 19) and GCCGGGCCCTATTTGTATAGTTCATCCATGC (SEQ ID NO: 20). Following standard procedures, we generated the different fusions by cloning PCR fragments of the respective genes into the C_{ub} and N_{ub} fusion vectors. The glutathione *S*-transferase (GST)-Nhp6B fusion was made by cloning the NHP6B ORF into GEX-5X-1 (Amersham Pharmacia). H_6HA-Tup1p was constructed by cloning a PCR fragment containing the TUP1 ORF, six histidines, and an HA tag into pET11a (Invitrogen).

Although Applicants believe no fees are due with the filing of this Preliminary Amendment, if there are any fees due, please charge the fees to our **Deposit Account No. 18-1945.** Please direct any questions arising from this submission to the undersigned at (617) 951-7615.

Respectfully Submitted,



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